Next generation sequencing in the context of current clinical practice: Implementation and challenges
Next generation sequencing of the cancer genome in the context of current clinical practice: Implementation and challenges
BIOLOGY OF CANCER

Networks of signal transduction pathways in the cell.

- Critical nodes ('hubs') in the signal transduction network are targets for oncogenic alterations

- Hanahan and Weinberg 2000/2011
Taming the dragon: genomic biomarkers to individualize the treatment of cancer
Nature Medicine 2011
# Targeted Therapeutics in Cancer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Alteration</th>
<th>Tumor Type</th>
<th>Therapeutic Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor tyrosine kinase</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td>Mutation, amplification</td>
<td>Lung cancer, glioblastoma</td>
<td>Gefitinib, erlotinib</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Amplification</td>
<td>Breast cancer</td>
<td>Lapatinib</td>
</tr>
<tr>
<td>FGFR1</td>
<td>Translocation</td>
<td>Chronic myeloid leukemia</td>
<td>PKC412, BIBF-1120</td>
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<tr>
<td>FGFR2</td>
<td>Amplification, mutation</td>
<td>Gastric, breast, endometrial cancer</td>
<td>PKC412, BIBF-1120</td>
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<tr>
<td>FGFR3</td>
<td>Translocation, mutation</td>
<td>Multiple myeloma</td>
<td>PKC412, BIBF-1120</td>
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<tr>
<td>PDGFRα</td>
<td>Mutation</td>
<td>Glioblastoma, gastrointestinal stromal tumor</td>
<td>Sunitinib, sorafenib, imatinib</td>
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<tr>
<td>PDGFRβ</td>
<td>Translocation</td>
<td>Chronic myelomonocytic leukemia</td>
<td>Sunitinib, sorafenib, imatinib</td>
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<tr>
<td>ALK</td>
<td>Mutation or amplification</td>
<td>Lung cancer, neuroblastoma, anaplastic large-cell lymphoma</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>c-MET</td>
<td>Amplification</td>
<td>Gefitinib-resistant non–small-cell lung cancer, gastric cancer</td>
<td>Crizotinib, XL184, SU11274</td>
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<tr>
<td>IGF1R</td>
<td>Activation by insulin-like growth factor II ligand</td>
<td>Colorectal, pancreatic cancer</td>
<td>CP-751,871, AMG479</td>
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<tr>
<td>c-KIT</td>
<td>Mutation</td>
<td>Gastrointestinal stromal tumor</td>
<td>Sunitinib, imatinib</td>
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<td>FLT3</td>
<td>Internal tandem duplication</td>
<td>Acute myeloid leukemia</td>
<td>Lestaurnitin, XL999</td>
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<tr>
<td>RET</td>
<td>Mutation, translocation</td>
<td>Thyroid medullary carcinoma</td>
<td>XL184</td>
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<tr>
<td><strong>Non–receptor tyrosine kinase</strong></td>
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<tr>
<td>ABL</td>
<td>Translocation (BCR-ABL)</td>
<td>Chronic myeloid leukemia</td>
<td>Imatinib</td>
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<tr>
<td>JAK2</td>
<td>Mutation (V617F), translocation</td>
<td>Chronic myeloid leukemia, myelo-proliferative disorders</td>
<td>Lestaurnitin, INCB018424</td>
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<tr>
<td>SRC</td>
<td>Overexpression</td>
<td>Non–small-cell lung cancer; ovarian, breast cancer; sarcoma</td>
<td>KX2–391, dasatinib, AZD0530</td>
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<td><strong>Serine–threonine–lipid kinase</strong></td>
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<tr>
<td>BRAF</td>
<td>Mutation (V600E)</td>
<td>Melanoma; colon, thyroid cancer</td>
<td>SB-590885, PLX-4032, RAF265, XL281</td>
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<tr>
<td>Aurora A and B kinases</td>
<td>Overexpression</td>
<td>Breast, colon cancer; leukemia</td>
<td>MK-5108 (VX-689)</td>
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<td>Polo-like kinases</td>
<td>Overexpression</td>
<td>Breast, lung, colon cancer; lymphoma</td>
<td>BI2536, GSK461364</td>
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<td>MTOR</td>
<td>Increased activation</td>
<td>Renal-cell carcinoma</td>
<td>Temsirolimus (CCI-779), BEZ235</td>
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<tr>
<td>PI3K</td>
<td>PIK3CA mutations</td>
<td>Colorectal, breast, gastric cancer; glioblastoma</td>
<td>BEZ235</td>
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<tr>
<td><strong>DNA damage or repair</strong></td>
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<tr>
<td>BRCA1 and BRCA2</td>
<td>Mutation (synthetic lethal effect)</td>
<td>Breast, ovarian cancer</td>
<td>Olaparib, MK-4827 (PARP inhibitors)</td>
</tr>
</tbody>
</table>

* PARP denotes poly(adenosine diphosphate–ribose) polymerase.
Daily practice

- Amplification
- Clonality
- Genomic alterations/CIN
- Mutation
- Methylation (CpG islands)
- MSI analysis
- Translocation
- (mi)RNA expression
- Ploidie status
- Typing

BRAF 1796T>A (V600E)

MSI Analysis
Current tools

- Allele specific assays
- Classic Sanger DNA sequencing
- Flow cytometry
- Fragment analysis (typing, MSI)
- FISH, CISH/BRISH
- LIPA
- MLPA
- Melting curve analysis
- RT-PCR
Challenges 2012-…. 

- Increasing numbers with: **Limited (pre-)operative material**
  - Early diagnosis, **Neoadjuvant therapies**
  - Only tissue biopsies/cores
  - Enrichment/microdissection steps
  - FNA material

- **Identification of tumorheterogeneity**

- Laboratory automation
Preoperative Staging: on Cytological material

- Current lung cancer staging guidelines acknowledge:
  - Endosonography with fine needle aspiration
    - Mediastinal lymph nodes
  - Minimally invasive alternative to surgical staging to detect nodal disease.
  - Minimal material

Pathological diagnosis: On Cytology from lymph nodes
Molecular Pathology on the same material: Option for Personalized medicine?

J. Annema et al JAMA. 2010;304(20):2245-2252
EGFR activated, KRAS wildtype
Limited material

FNA mediastinal lymphnode
Allele specific taqman probes = FAST
Rapid Mutation Analysis of Fine Needle Aspirates using allele-specific qPCR

- EGFR: p.L858R*, exon 19 deletions*
- NRAS, HRAS, KIT, IDH1/2, ….
And it works on minimal input DNA

Effect of the DNA concentration on the c.34G.T KRAS assay.

R. van Eijk et al PlosONE 2011: 6 (3) e17791
And it works on minimal input DNA

Initial analysis in Bio-Rad CFX Manager
EGFR deletion exon 19

‘Classical detection’
FAST assay for exon 19 deletions

Wild type signal is lost if a deletion is present


Tony K.F. Yung,1,2 K.C. Allen Chan,1,2 Tony S.K. Mok,1,3 Joanna Tong,4 Ka-Fai To,4 and Y.M. Dennis Lo1,2,5


Rapid KRAS, EGFR, BRAF and PIK3CA Mutation Analysis of Fine Needle Aspirates from Non-Small-Cell Lung Cancer Using Allele-Specific qPCR

Tumor heterogeneity in melanoma

BRAFV600E

COBAS: BRAFV600 +

NO BRAF V600E

GTG -> AAG: V600K

2 Subcutane lesions
Intra- and Inter-Tumor Heterogeneity of $BRAF^{V600E}$ Mutations in Primary and Metastatic Melanoma

Molly Yancovitz$^{1,9}$, Adam Litterman$^{1,9}$, Joanne Yoon$^{1}$, Elise Ng$^{1}$, Richard L. Shapiro$^{2}$, Russell S. Berman$^{2}$, Anna C. Pavlick$^{1,3}$, Farbod Darvishian$^{4}$, Paul Christos$^{5}$, Madhu Mazumdar$^{5}$, Iman Osman$^{1,3}$, David Polsky$^{1,4,6}$

### Table 4. BRAF mutation concordance between primary and metastatic specimens using MS-PCR.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary tumor</th>
<th>Metastatic tumor</th>
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<tr>
<td>1</td>
<td>Wild Type</td>
<td>Mutant</td>
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<td>2</td>
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<tr>
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<td>7</td>
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<td>17</td>
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<tr>
<td>18</td>
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<td>Wild Type</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0029336.004
Tumor heterogeneity 1

Tumor heterogeneity 2: A trunk-branch model of intratumor heterogeneity.


Charles Darwin: The origin of species
NGS current vs NGS limited material

- FFPE biopsies /FNA
- Mutoom Genomic, Methylomic, Transcriptomic, miRNA, fusion info needed.
- No DNA/cDNA amplification possible because of false positivity
NGS first/2nd/third

- FFPE Sequenom
- FFPE Target enrichment of selected gene sets
- Helicos
- Illumina high seq/my seq
- Ion/Proton Torrent
- Nanopore
- Complete genomics

Challenges:
- Frozen tissue vs FFPE
- False positivity rates?
  Bioinformatics
- Logistics?
Taming the dragon: genomic biomarkers to individualize the treatment of cancer
Nature Medicine 2011
Concluding remarks:

Improving the human condition with genomic medicine

http://personalgenome.com/

Read Vogelstein and Kinzler:

Winning the war: Science Parkour

There is a difference between *proclaiming* a war and *winning* a war. In 1971, U.S. President Richard Nixon proclaimed a war on cancer.

Prevention and early detection

Immunotherapy, targeted therapy, achieving victory.

Hanahan and Weinberg, 2011